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Morphological Effects of Low Temperatures during the Embryonic Development of the Garter Snake, *Thamnophis elegans*[1]

WADE FOX, CHARLES GORDON[2] & MARJORIE H. FOX

Louisiana State University, School of Medicine and Department of Biology, New Orleans

(Text-figures 1-4)

SINCE SCUTE PATTERNS and scute ("scale") counts are among the principal taxonomic characteristics of reptiles, any experimental evidence suggesting that scutellation can be altered by environmental factors is of considerable interest to herpetologists. One of us (Fox, 1948a) reported one such experiment in which exposure to low temperatures during embryonic development resulted in decreased numbers and modified patterns of scutes in garter snakes. If such accidents of development can happen in the laboratory, conceivably they can also occur under natural conditions.

Although the data presented in 1948 were statistically significant, they were not altogether convincing because of the small number of surviving experimental litters and the small size of these litters. Hence, the experiment was repeated in 1948 under essentially the same laboratory conditions as in 1947 (reported in 1948).

METHODS

The subspecies of garter snake used in this experiment was the same as was used in the previous report. This was referred to as *Thamnophis elegans atratus* according to the designated classification at that time (Fox, 1948b). However, this population of garter snakes was of the terrestrial form which later (Fox, 1951) became known as *Thamnophis elegans terrestris.* None of the snakes used was of the semi-aquatic type which Fox (1951) recognized as a distinct although sympatric race of garter snake identical to the type specimens of *Thamnophis elegans atratus* (Kennicott).

The garter snakes, *Thamnophis elegans terrestris,* were collected from a 25-mile stretch of the Pacific coast which included the 1947 collecting site on Skyline Blvd. of the San Francisco Peninsula in San Mateo County, California. Since the Skyline Blvd. area had been collected heavily during the previous three years, a more extensive collecting area proved necessary in order to find adequate numbers of gravid female snakes. All animals were collected during the last week of April and the first week of May as it had been determined from previous collecting that gravid females obtained during this period would already be inseminated and would either have ovulated recently or would probably ovulate within a few days following capture.

Immediately upon capture 18 gravid females were placed in a cool room (experimentals) in which the temperature ranged from 65° to 85° F, and four gravid females were placed in a warm room (controls) in which the temperature ranged from 75° to 95°F, the same temperature ranges utilized in 1947. These rooms were located in a small, incompletely insulated greenhouse and the temperature variations reflected variations in the outside environmental temperatures. The temperature rose and fell simultaneously in both rooms but a marked differential was maintained between them. The small control sample seemed permissible in view of the very large control sample available from the previous experiment (1947) and the desirability of making the experimental sample as large as possible.

The four control females littered 46 young in early August. By September the experimental mothers had not littered and it was decided to terminate the experiment. The mothers were killed and fetuses were obtained from 15 of them. The others contained partially reabsorbed

[1]Experiment conducted at the University of California, Berkeley.

[2]Partially supported by a grant from the Louisiana Heart Association.

TABLE I

	Samples	Sample Size	Ventrals	P[3]	Subcaudals	P[3]
1	♀ 1948 Experimental	63	150.3 ± 10.0[1] 138 – 160[2]		65.6 ± 9.3[1] 48 – 77[2]	
2	Mothers of 1948 Experimental	12	154.3 ± 3.9 149 – 160	.03	71.2 ± 4.0 67 – 80	.005
3	♀ 1948 Control	20	154.5 ± 3.5 150 – 158	.008	73.7 ± 3.8 68 – 76	<.001
4	♀ 1947 Control	50	154.9 ± 4.1 147 – 167	.005	70.4 ± 2.9 62 – 77	.001
5	♂ 1948 Experimental	65	154.6 ± 11.0 134 – 163		74.7 ± 11.2 48 – 86	
6	♂ 1948 Control	26	160.8 ± 3.4 154 – 164	<.001	82.7 ± 6.5 71 – 89	<.001
7	♂ 1947 Control	58	158.7 ± 3.6 152 – 168	.007	80.5 ± 3.7 71 – 88	<.001

[1] Mean and standard deviation.
[2] Range.
[3] Probabilities of significance of differences between the female experimental sample and their mothers and two female control samples, and between the male experimental sample and the two male control samples.

embryos and hard masses of yolk in their oviducts. The experimental fetuses were in various stages of maturity but all had fully completed the development of scute patterns. The young snakes and their mothers were preserved in alcohol or formalin and stored in jars until 1958. During the interval three of the litters dried out so badly that reliable scale counts could not be made. Therefore the expermental sample is based on 12 litters yielding 128 fetuses. In a few other instances the number of scale rows around the body was questionable due to dehydration of the specimens. These were also omitted from the calculations.

The number and condition of the following scutes were observed on each specimen: ventrals, subcaudals, longitudinal rows around the body, upper labials, lower labials, preoculars, postoculars, temporals, loreals, nasals, chin shields and anal plate. Special abnormalities were noted wherever they appeared.

The significance of differences between the experimental litters and (1) their mothers, (2) the 1948 controls, and (3) the 1947 controls were calculated (Tables I, II, III, and IV). Males were tested against males and females against females for all scute counts. Within the experimental samples and the control samples the litters were pooled and no allowance was made for variation between litters. In cases of bilateral characteristics, left and right sides were tested independently. Student's *t* test was used in calculating the significance of the differences in numbers of ventral and subcaudal scutes.[3] All other characteristics were tested by formula for chi-square (including Yates' correction factor). Probability values were taken from Fisher's tables.

RESULTS

Ventrals and Subcaudals.—The female experimental fetuses (Table I, Row 1) had significantly fewer ventral and subcaudal scutes than their mothers (Row 2), the females of the 1948 controls (Row 3) and the females of the 1947 controls (Row 4). The mean numbers of ventrals and subcaudals of the male experimental sample (Row 5) were very significantly smaller than those of the 1948 (Row 6) and 1947 (Row 7) controls.

Seven litters averaged significantly lower in numbers of ventrals and/or subcaudals than the averages of the control samples or the natural population (Text-figs. 1 and 2). Two litters averaged approximately the same as the latter averages, and three litters averaged slightly high-

[3] A comment is in order concerning the assumption of homogeneity of variance in the groups under study. The hypothesis that the variances were equal in the two populations of experimentals was accepted; likewise, the hypothesis that the variances were equal in the five control groups was accepted. The hypothesis that the variances were equal in all seven groups was rejected. Therefore, when comparisons are made between the experimental and control groups by means of the *t*-test the assumptions of the *t*-test are not met. In spite of the above, the approximate *t*-test was used and the appropriate number of degrees of freedom estimated.

er than the control averages. Extreme reductions in scute numbers occurred in six of the experimental litters. In one of these the four males averaged 137.8 ventrals and 57.3 subcaudals, whereas the lowest averages for males in a single control litter were 155.4 and 75.6 respectively. The lowest litter averages for female experimental fetuses were 143.7 ventrals and 54.3 subcaudals, whereas the lowest averages for a control litter were 153.0 and 68.1 respectively.

It is of interest to compare each litter with its respective mother. Ventrals (Text-fig. 1) in the female fetuses of eight experimental litters and subcaudals (Text-fig. 2) in six were markedly lower than the numbers of these scutes on their respective mothers. In four litters the averages of the females were more or less equal to those of their respective mothers, but in no case was the litter average markedly higher than that of the mother. Additionally, in litter No. 10 (Text-fig. 1) the two surviving female fetuses were normal but three of the four males possessed the smallest number of ventrals encountered.

Scale Rows.— The numbers of longitudinal scale rows on the body were counted at the neck, thoracic region and at the caudal end of the body just in front of the vent. In using chi-square to test the differences between samples, frequencies of occurrence of 20 scale rows or less were tested against frequencies of 21 rows or more in the neck and thoracic regions; for the caudal end of the body frequencies of 16 rows or less were tested against 17 or more.

This race of garter snake usually has 19 scale rows at the neck. No wild populations in the range of the subspecies were found with more than 21 rows and only in the northernmost populations were a few individuals found with fewer than 19 rows (Fox, 1948a). The majority of experimental males and females possessed 19 scale rows at the neck (Table II, Rows 1 and 5) but three had only 17 rows, one had 22 and two had 23. Hence, animals exposed to cool temperatures had a wider range of variation than did the control samples. The number of scale rows at the neck of the mothers of the experimentals did not differ significantly from those of their female offspring (Table II). The six individuals with extremely low (17) or extremely high (22, 23) numbers of scale rows were all born to mothers with 19 scale rows.

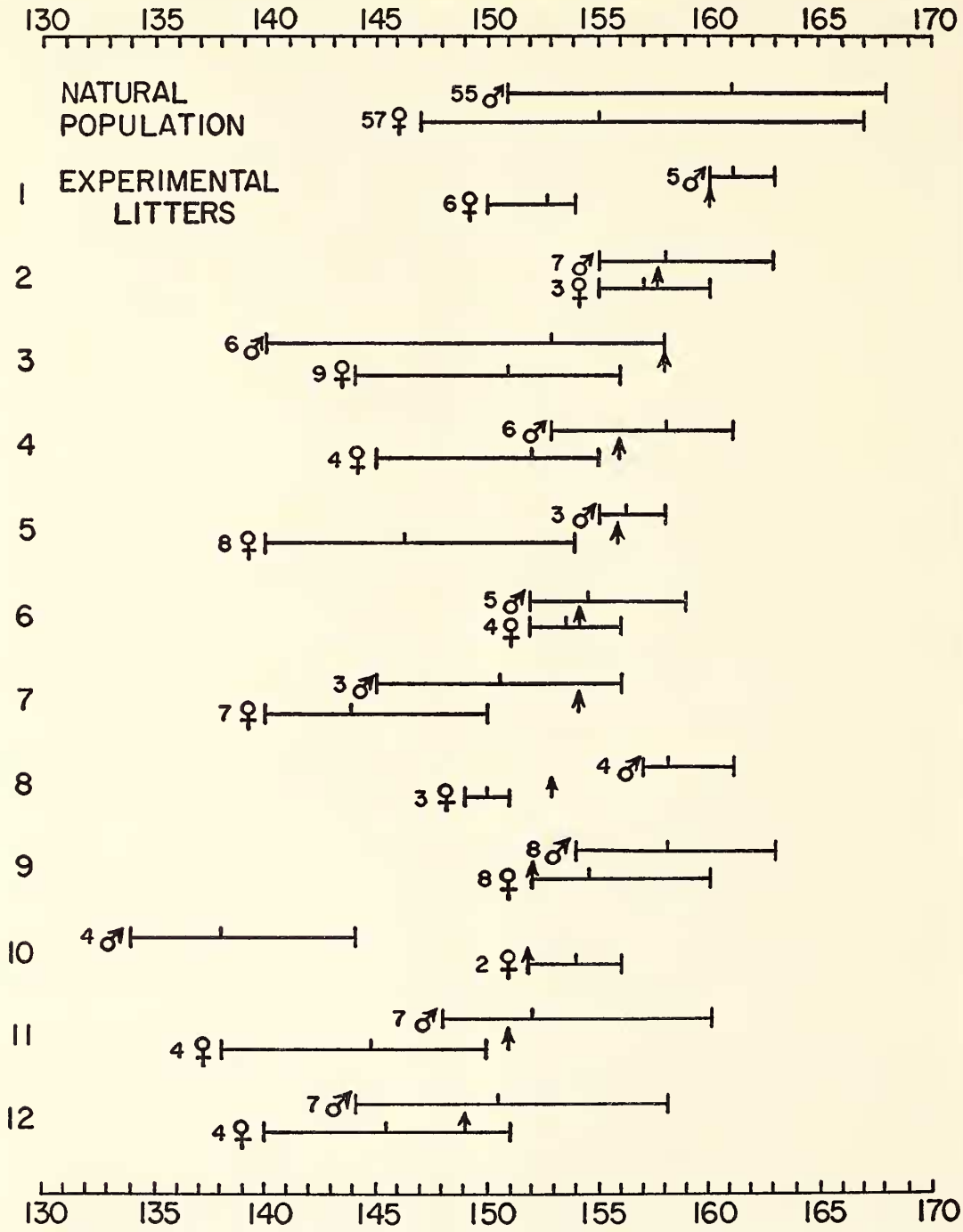
A peculiar inconsistency appeared in the 1948 study. The controls, both males and females, showed a high tendency for 21 scale rows at the neck, whereas nearly all the controls of the 1947 experiment had 19 rows. This resulted in a very significant P value when comparing the 1948 experimentals with the 1948 controls. However, when comparing the 1948 experimentals with 1947 controls no significant differences were found between the females, and the experimental males revealed an unexpected significantly greater tendency towards an increased number of scale rows (Table II).

There are 21 scale rows in the thoracic region (the usual position of the maximum number) in about 75 per cent. of the wild population (Fox, 1948a). The 1948 experimentals showed a considerably greater tendency toward a reduction to 19 scale rows than did their mothers, the 1947 or 1948 controls (Table II), or the wild population. A surprisingly large number of the experimental males had only 17 scale rows in the thoracic region, whereas no count this low was found among the controls or wild population. Nearly all individuals with 17 scale rows were born to mothers with 21 rather than 19 scale rows. This suggested that the experimental conditions played a more significant role in determining fetal scutellation than did the maternal pattern. The 1948 male controls displayed a greater tendency toward 21 scale rows in the thoracic region than did the 1947 male controls.

As in almost all specimens from the natural population in the area of collection, 17 rows of scales were present at the caudal end of the body of nearly all controls. Although there was a greater tendency toward reduction of this number in the experimentals, this trend did not test to be significant (Table II). Nearly all of the individuals showing a posterior reduction of scale rows were from four litters. One of these litters included one individual with 14 rows, seven individuals with 15, one with 16 and two with 17. The mother of this litter had a typical scale formula: 19-21-17. An unexpectedly large number of male experimentals with 18 or 19 scale rows occurred in three experimental litters.

Labials.—In *T. e. terrestris the* customary number of upper labials is 8 (Text-fig. 3A), but a tendency for this number to be reduced to 7 (Text-fig. 3B) has been found in populations from cooler climates (Fox, 1948a). Male and female experimental fetuses were considerably more variable (Table III) in this characteristic than the wild sample. In spite of the occasional occurrence of nine upper labials, the male and female experimental samples had significantly fewer upper labials (Text-fig. 3C, D, E, F) on both left and right sides than either their mothers, control fetuses or the natural population from the area of capture.

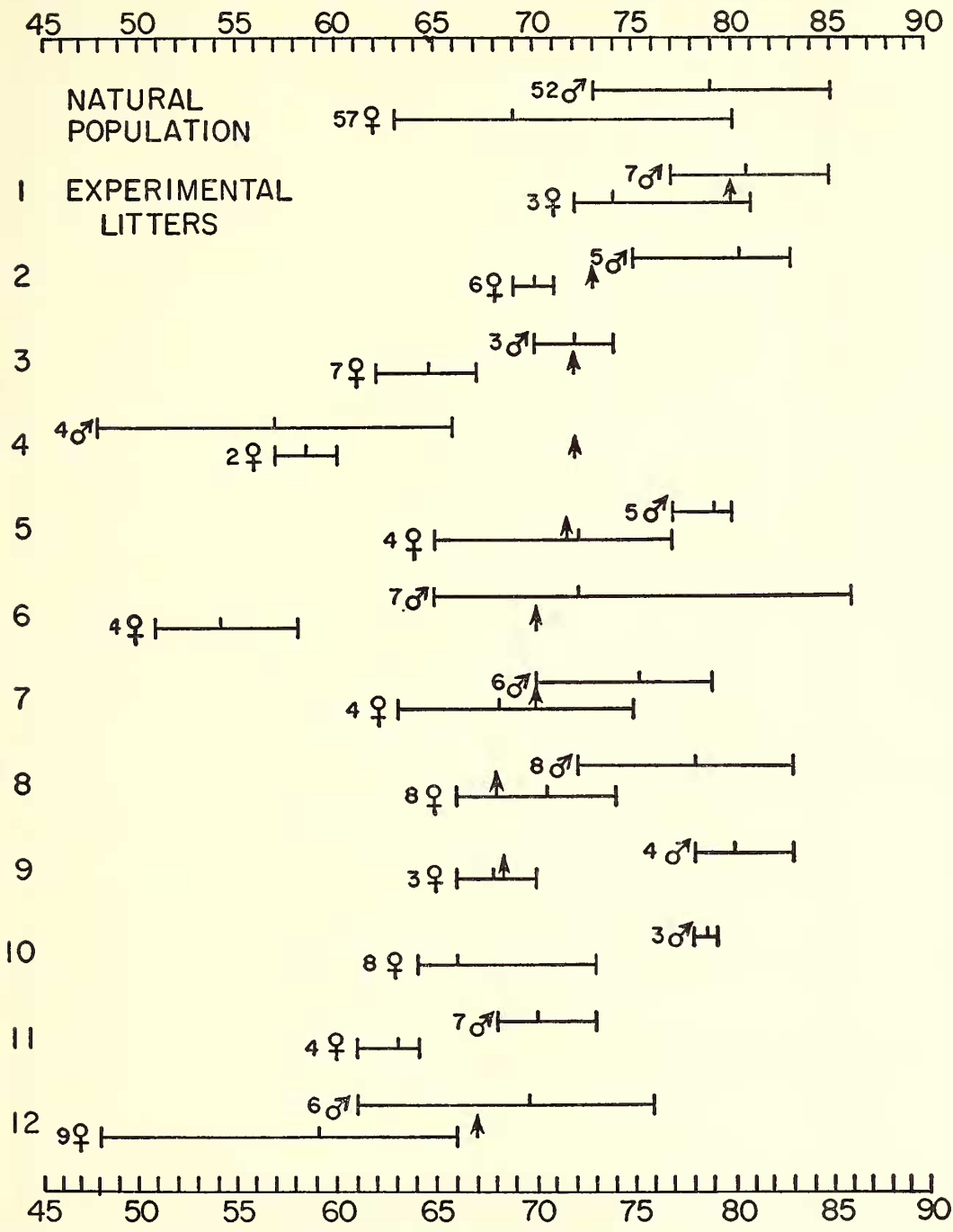
Ten lower labials were present in 82 per cent. of the natural population. As with the upper labials, although this normal scale number was



TEXT-FIG. 1. Range and mean number of ventral scutes found in the 12 experimental litters and compared with the natural population from the San Francisco Peninsula. The number of ventrals of each mother is indicated by the vertical arrows which are arranged in increasing order from bottom to top.

occasionally exceeded by a few experimental fetuses, the strongest trend was towards a significant reduction (Table III; Text-fig. 3B, C, F; Text-fig. 4B).

Most individuals of all but three litters possessed a reduced number of upper and lower labials. Of these three litters, one contained an unusually high number of individuals with 11



TEXT-FIG. 2. Range and mean number of caudal scutes found in the 12 experimental litters and compared with the natural population. The number of caudals of each mother is arranged in increasing order from bottom to top. Mothers of litters of 10 and 11 had incomplete tails.

lower labials (Text-fig. 1E). The other two litters possessed the characteristic number of labials, in spite of the fact that the mother of one of these litters had a partially reduced number of labials, *i.e.* 8-7 upper labials and 10-9 lower labials. Among the litters with reduced numbers of labials was one whose mother possessed a bilateral reduction of lower labials (9-9). Eighty-

TABLE II. FREQUENCIES OF NUMBER OF SCALE ROWS

Samples		14	15	16	17	18	19	20	21	22	23	P[1]
1 ♀ 1948 Experimental	Neck				2		33	3	16		1	
2 Mothers 1948							9		3			.90
3 ♀ 1948 Control								1	16	3		<.001
4 ♀ 1947 Control							37	5	8			.90
5 ♂ 1948 Experimental					1		38	3	14	1	1	
6 ♂ 1948 Control								1	22	3		<.001
7 ♂ 1947 Control							53	3	2			.005
1 ♀ 1948 Experimental	Thoracic Region				1		16	7	27	3		
2 Mothers 1948							3		8	1		.30
3 ♀ 1948 Control								1	19			.007
4 ♀ 1947 Control								1	49			<.001
5 ♂ 1948 Experimental					8	1	19	5	24	1		
6 ♂ 1948 Control									25	1		<.001
7 ♂ 1947 Control							11	3	44			.001
1 ♀ 1948 Experimental	Caudal end of Body		3	5	44	2						
2 Mothers 1948					11	1						.15
3 ♀ 1948 Control					20							.12
4 ♀ 1947 Control			1	3	46							.56
5 ♂ 1948 Experimental		1	7	7	35	7	1					
6 ♂ 1948 Control					26							.22
7 ♂ 1947 Control			3	4	51							.15

[1] Probabilities of significance of differences between the female experimental sample and their mothers and two female control samples, and between the male experimental sample and the two male control samples.

five per cent. of the lower labial scale counts of fetuses in this litter were found to be nine. This number was found on either right or left side or bilaterally. The remaining 15 per cent. of the scale counts showed a reduction to eight lower labials or the normal number of ten. Eight labials occurred either bilaterally or unilaterally, but ten occurred only unilaterally. No example of extreme reduction occurred in this litter. All of the cases of extreme reduction in number of upper and lower labials occurred in litters of mothers with the characteristic number of labials.

Preoculars and Postoculars.—Although a single preocular scute is characteristic of the race (Text-fig. 3A), even in natural populations an occasional specimen is found in which this scute

TABLE III

	Samples		Upper Labials							Lower Labials							Preoculars					Postoculars				
			4	5	6	7	8	9	P[1]	7	8	9	10	11	P[1]		1	2	3	P[1]		1	2	3	4	P[1]
1	♀ 1948 Experimental	L		8	10	14	30	1			20	26	15	2			44	18	1			3	40	16	4	
		R	4	13	21	23	2				23	24	16				40	22	1			6	29	24	4	
2	Mothers 1948	L				1	11		.01			2	10		<.001		11	1		.20			2	10		.001
		R				1	11		.002			2	10		<.001		11	1		.10			1	11		.005
3	♀ 1948 Control	L				3	17		.01			1	19		<.001		20			.017			3	17		<.001
		R				1	19		<.001			2	18		<.001		20			.006		2	17	1		<.001
4	♀ 1947 Control	L				5	45		<.001			1	49		<.001		47	3		.006			5	45		<.001
		R				5	44	1	<.001			3	44	3	<.001		49	1		<.001			2	48		<.001
5	♂ 1948 Experimental	L		6	16	10	28	5		2	17	25	20	1			38	26	1			1	33	29	2	
		R	2	4	9	19	28	3		1	18	28	16	2			41	24				1	34	28	2	
6	♂ 1948 Control	L				1	25		<.001			2	24		<.001		26			<.001			1	24	1	<.001
		R				1	24	1	<.001			2	24		<.001		26			.001			2	22	2	.001
7	♂ 1947 Control	L				10	48		<.001			3	55		<.001		51	7		<.001			5	53		<.001
		R				5	53		<.001			2	56		<.001		55	3		<.001			3	55		<.001

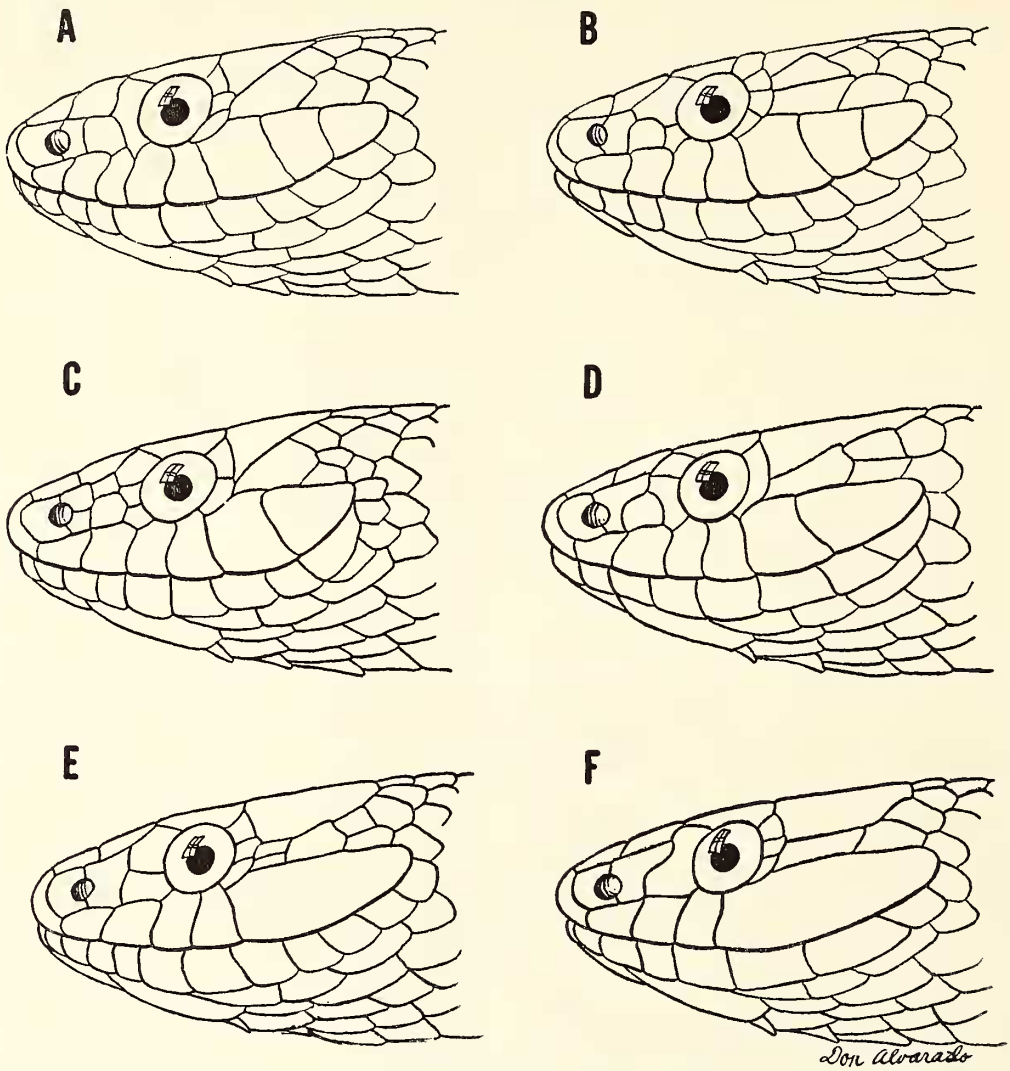
[1] Probabilities of significance of differences between the female experimental sample and their mothers and two female control samples, and between the male experimental sample and the two male control samples. L (left) and R (right) sides tested separately.

is horizontally divided about one-third of the distance from the bottom. The cool room treatment resulted in a high proportion of preocular scutes being divided into two scutes (Text-fig. 3B, and D) and rarely into three scutes (Table III, Text-fig. 3C).

In natural populations postoculars usually appear as three separate scutes (Text-fig. 3A). However, the skin bordering the eye posteriorly is occasionally fused into only two separate scutes or rarely further divided to form four separate scutes. This trend towards deviation from the typical pattern of three scutes was very pronounced in the experimental litters. Four postoculars (Text-fig. 3B) occurred more frequently than in natural populations and the tendency towards reduction of the number of scutes to two (Text-fig. 3C) or even one (Text-fig. 3D) was far greater than was found in the mothers, the controls (Table III) or natural populations. In two specimens the uppermost postocular was fused with an upper first temporal.

Several of the experimental mothers exhibited atypical patterns of preoculars and postoculars (Table III). Their offspring did not appear to display a significantly greater tendency to deviate from the characteristic pattern of these scutes than did the offspring of mothers with typical patterns. Of the 12 litter mothers, six had an atypical number of preoculars or postoculars on one side and six had typical numbers on both sides. It is difficult to demonstrate that the ocular pattern of the mothers determined the pattern of the fetuses. However, the litter with the largest proportion of fetuses with characteristic ocular scute patterns (13 out of 17) was born to a mother with the characteristic pattern. On the other hand, a litter of nine whose mother possessed two preoculars on both sides contained five fetuses with one preocular on both sides, four with one on one side and two on the other, but none with the pattern of the mother.

Temporals.— The arrangement of temporal scutes also proved to be very labile. A number of different patterns are indicated in Table IV and Text-fig. 3. The arrangement 1-2-3 (Text-fig. 3A) is the most common pattern in this species. Much deviation was seen in wild populations, in the mothers of the experimentals and in the control fetuses (Table IV). In order to use the method of chi-square to test the significance of the differences, the frequencies of the pattern 1-2-3 were treated as the expected and all other variations were pooled as deviations from the expected. As can be seen from the P values in Table IV, the experimental samples proved to have a significantly greater deviation



TEXT-FIG. 3. Variations in scute patterns on side of head. **A.** Typical pattern: 1 preocular, 3 postoculars, 1 postnasal, 1 loreal, 8 upper labials, 10 lower labials, temporals 1-2-3. **B.** Postnasal and upper preocular meet, preocular divided below, 4 postoculars, 7 upper labials, 9 lower labials, temporals 2-2-3. **C.** Postnasal divided, 2 loreals (upper and lower), 3 preoculars, 2 postoculars, 6 upper labials, 8 lower labials, temporals 1-3-3. **D.** Postnasal fused to upper loreal, preocular divided above, 1 postocular, 6 upper labials, 7 lower labials (first fused to mental), temporals 1-2-2. **E.** Lower loreal fused to lower preocular, 3 postoculars, 5 upper labials, 11 lower labials, temporals 2-1-2. **F.** Upper loreal fused to postnasal, lower loreal fused to lower part of preocular, 4 upper labials, 7 lower labials, temporals 1-1-2.

from the expected than did the controls. When the left and right sides of the experimental female fetuses were compared to the respective sides of the mothers, the level of significance was border line (Table IV, Row 2); however, when left and right sides of the samples were pooled the difference proved to be very significant ($P = .007$). Variation of this characteristic was so great among the experimental fetuses that there was no clear correlation between the patterns of the fetuses in each individual litter and the pattern of the respective mothers.

Anal Plate.—The anal plate, which is normally undivided in the genus *Thamnophis*, was found to be divided in ten male and nine female experimental fetuses and grooved or notched in seven others. All of these fetuses came from five litters. Divided or grooved anal plates were not observed in the controls, the mothers or the natural populations of this race. However, Tanner

TABLE IV

	Samples		Temporals															Anal Plate	
			1-1-2	1-1-3	1-2-2	1-2-3	1-2-4	1-3-2	1-3-3	1-3-4	2-1-2	2-1-3	2-2-2	2-2-3	Others	P[1]	Divided	Undivided
1	♀ 1948 Experimental	L		2	8	23	1		3	1	1	1	1	5	17		9	54
		R	3	4	9	20	1	1	2	1			3	3	16			
2	Mothers 1948	L			3	9										.02	0	13
		R	1		2	8			1							.05		
3	♀ 1948 Control	L				15			4					1		.008	0	20
		R				19			1							<.001		
4	♀ 1947 Control	L			3	40	3		1					1	2	<.001	0	50
		R			4	39	2	1	1						3	<.001		
5	♂ 1948 Experimental	L	4	2	8	27			3		2	1			18		10	55
		R	3	3	8	20	1		5	2				1	22			
6	♂ 1948 Control	L				19	3		4							.009	0	26
		R				16	4	1	4	1						.015		
7	♂ 1947 Control	L	1	1	12	42			2							.001	0	58
		R			4	51	1								2	<.001		

[1] Probabilities of significance of differences between the female experimental sample and their mothers and two female control samples, and between the male experimental sample and the two male control samples. L (left) and R (right) sides tested separately.

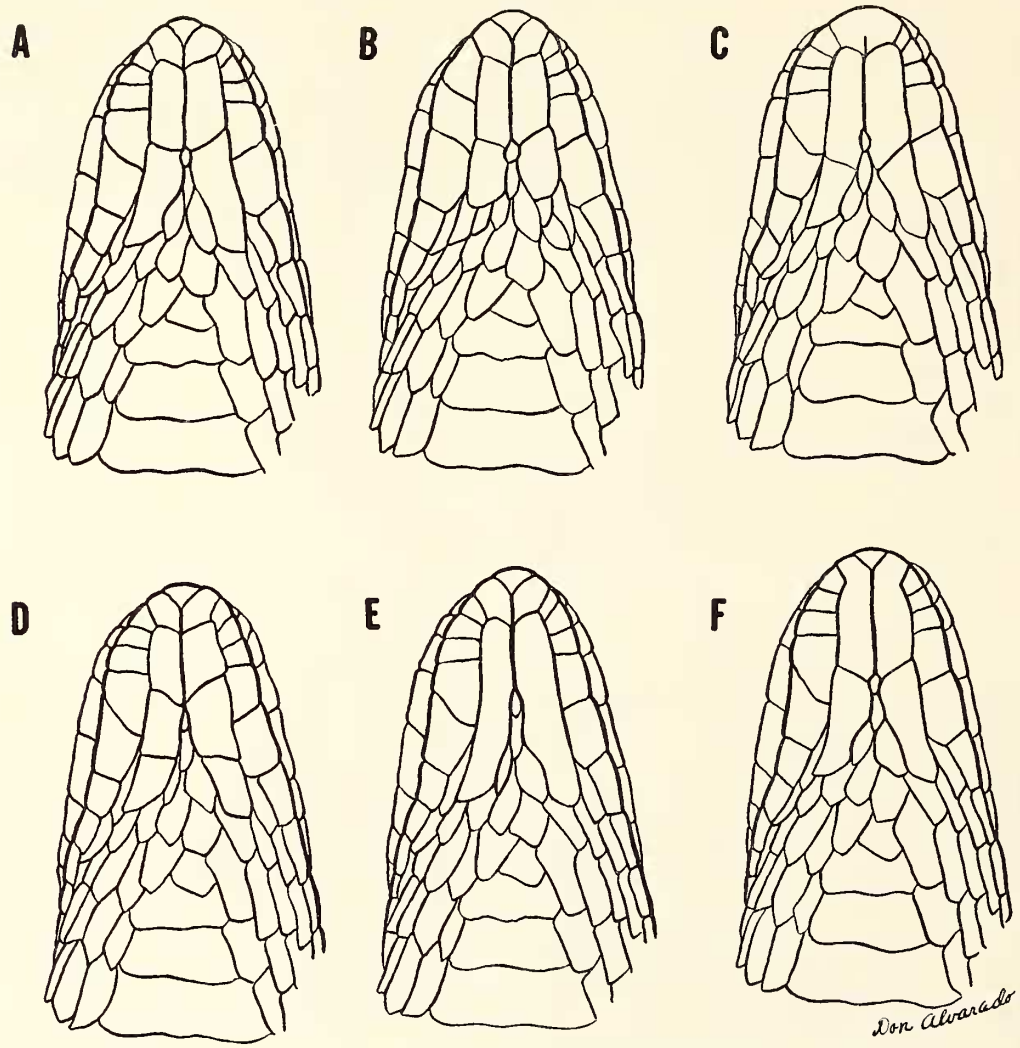
(1950) reported the occurrence of several specimens of another race of this species (*Thamnophis elegans vagrans*) from several populations in Utah with divided or grooved anal plates.

Chin Shields.—The same litters in which divided anal plates occurred showed considerable variation in the chin shields (Text-fig. 4). The posterior chin shields were frequently unusually short (Text-fig. 4B, C), whereas the anterior chin shields were occasionally exceptionally long (Text-fig. 4C). In three individuals the chin shields were divided into three pairs instead of the normal two pairs (Text-fig. 4D). In two others the anterior and posterior chin shields were fused (Text-fig. 4E), resulting in a single chin shield on one side in one instance and paired single chin shields in the other. Anterior chin shields were fused to the first labials in another specimen (Text-fig. 4F).

Loreals, Postnasals and Postrostrals.— Typically a single loreal scute separates the postnasal and preocular scutes (Text-fig. 3A). Only three experimental litters displayed variations from this condition. However, out of 31 individuals in these litters only one was completely normal in this respect and only two were merely unilaterally malformed. The remaining 28 fetuses had bilateral abnormalities of various types. In four individuals the preocular and postnasal made contact above the middle of a single loreal (Text-fig. 3B). In most others there were two loreals, one on top of the other (Text-fig. 3C). The upper loreal appeared to be derived from the lateral portion of the prefrontal. In five individuals this upper loreal was fused to the postnasal (Text-fig. 3D) on one or both sides of the head. In two individuals the lower loreal was fused to the lower preocular on one side (Text-fig. 3E). Peculiar fusions in one individual left the lower loreal fused to the lower preocular and the upper loreal fused to the postnasal (Text-fig. 3F). In one individual two lower loreals were present, but no upper. Postnasals were occasionally divided (Text-fig. 3C).

In one experimental litter three individuals out of ten possessed a single small median postrostral scute. This characteristic was not noted in any other garter snakes. In three individuals from two litters the mental scale was fused to the first lower labial: two on one side of the jaw only and one on both sides (Text-fig. 4C).

Harelip.— Three experimental litters contained individuals in which the naso-labial groove had failed to close during embryonic development. This condition resembled the harelip of mammals. In one litter of 11, two individuals retained open naso-labial grooves on both sides and



TEXT-FIG. 4. Variations in scute patterns on ventral surface of head. **A.** Typical pattern: 2 pair chin shields, 1 mental, 10 lower labials. **B.** Posterior chin shields short, 9 lower labials. **C.** Anterior chin shields unusually long, first lower labials fused to mental. **D.** Three pair of chin shields. **E.** One pair of chin shields. **F.** First lower labials fused to anterior chin shields.

three on one side only. In a second litter of six the cleft was present on both sides in two and one side in one. In the third litter of 11 one fetus retained a cleft on one side. Of the nine individuals with this malformation, seven were males and two were females.

DISCUSSION

On the basis of admittedly limited data, Fox (1948a) suggested that scutellation patterns of garter snakes could be influenced by lowering the environmental temperature during the gestation period. The larger number of significantly affected experimental litters found in the current study completely confirms this hypothesis and leaves little doubt that cool temperatures during embryonic development do result in an alteration of scute patterns. The principal effect observed was a reduction in the number of ventrals, subcaudals, labials and scale rows around the body; but all scute patterns underwent a marked increase in variability. There was no indication that ventrals or subcaudals were increased in any of the experimental litters; those experimental litters with higher numbers of these scutes fell well within the range of the controls (Table I) or the natural population (Text-fig. 1). There was a slight suggestion of increase in

numbers of scale rows around the body. However, only in the case of the number around the caudal end of the body does there appear to be a possibly significant increase (Table II), and this would be difficult to prove statistically.

A few experimental animals appeared to have an extra upper or lower labial. This extra labial was less frequently present in the wild population or the controls. However, since the evaluation of the presence of an extra labial is very subjective, it does not appear wise to emphasize strongly the few cases where the number of labials were increased. Clearly, the major effect of cool temperatures was a reduction in the number of labials. Scale characters such as preoculars, postoculars, temporals, loreals and chin shields are probably best described as showing a great deal of variation in their response to chilling. Many patterns resulting from atypical fusions or divisions of scutes appeared among the experimentals.

Of the twelve experimental litters reported on in this paper, eight showed significant modifications of most scale patterns analyzed, while the remaining four showed more variations in certain scale patterns than did the control litters. As can be seen in Text-figs. 1 and 2 and the Tables, not only individual litters but the means and the ranges of the pooled experimental litters differed very significantly from those of the control samples, the natural population and the litter mothers in most characteristics measured.

In addition to the fact that the temperature differential between the cold and warm rooms clearly influenced the pattern of fetal scutellation, temperature fluctuations within the rooms themselves may have played a significant role in influencing the outcome of the experiment. Fluctuations in the cool room may have been responsible for the survival of the experimental fetuses. We have been informed by Dr. James A. Oliver of the American Museum of Natural History (personal communication) of similar experiments in which snake eggs were maintained at a constant low temperature. These experiments resulted in the death of all cold-exposed embryos. He suggests that our success may have been due to the fact that the temperature fluctuations in our cool room (65°-85°F) resulted in the animals being exposed to lethal temperatures for only part of the day, the remainder of the time being spent at temperatures compatible with morphogenesis and survival. His explanation seems plausible, for even with our fluctuating temperatures there was a high mortality in the 1947 experimental series and in the more successful 1948 series three entire litters died.

Although it is clear that environmental temperature can influence the scutellation pattern of developing garter snakes, the actual mechanism by which it controls scute development is unknown. The data indicate that the fluctuating temperatures of our experimental rooms did not influence all scale characters in the same way. Ventrals, subcaudals and scale rows in the thoracic region, if affected at all, were invariably reduced in number, whereas preoculars and anal plates were subdivided, resulting in increased scale counts. Further, individual characters were not uniformly affected in different embryos. Fetuses maintained in the cool room usually showed a marked reduction in the number of labials and scale rows around the body in the neck region, yet there were more fetuses than usual in which these scale numbers were increased. Individual responses were particularly marked in such scutes as postoculars, temporals, loreals and chin shields where lowered temperatures lead to a wide range of patterns resulting from subdivision, lack of division, abnormal growth or fusion of scutes.

This multiplicity of reactions to lowered temperatures might be anticipated because studies of developing turtle (Yntema, 1960) and fish (see review by Tåning, 1952) embryos indicate that in poikilothermic vertebrates the morphogenetic effects of temperature variations depend on a number of factors. In the most comprehensive investigation of this type, Tåning (1952) demonstrated that modifications of individual meristic characters of embryos of the sea-trout, *Salmo trutta trutta,* varied independently with the exact degree of temperature applied. At constant temperatures between 2.7°C and 14°C the number of vertebrae was lowest at 6°C and rose at higher or lower temperatures. Curves for the number of fin rays, however, were almost the reverse of that for the vertebrae since the former reached their maxima between 6°C to 10°C and fell off at higher or lower temperatures. Tåning further demonstrated that temperature variations affected the morphogenesis of the vertebrae differently at different stages of development. During an early sensitive period from fertilization to gastrulation the temperature effect was as stated above, namely a decrease in vertebral number with a temperature drop from 10°C to 4°C. During a later "supersensitive" period, just before the last vertebrae were preformed as posterior mesodermal segments, the temperature effects were reversed. At this time a drop from 10°C to 4°C resulted in a marked increase in the number of vertebrae. Lastly, this study indicated that while genetical differences were important in determining vertebral modi-

fications, fin ray variations appeared to be almost purely phenotypic.

Tåning's findings are of particular interest because if the thermal reactions of developing garter snakes are similar to those of the sea-trout, many puzzling aspects of our data can be clarified. The consistent ratio of 1 vertebra: 1 pair of ribs: 1 ventral scute which pertains in snakes made us suspect that modifications in the number of these scutes represented a basic vertebral and somite disturbance. Examination of eight stained and cleared experimental fetuses with reduced numbers of ventral scutes confirmed our suspicions. With very few exceptions, one ventral scute was associated with one pair of ribs and one vertebra. With the possible exception of the terminal scute, the subcaudals and vertebrae also appeared to maintain a 1:1 ratio. The consistent reduction of ventrals and subcaudals in our experimental samples is therefore in keeping with the data of Tåning (1952), Schmidt (1921), and Lindsey (1954), who demonstrated that, down to a certain critical point, low temperatures usually resulted in a reduction of vertebrae in fish embryos and with those of Yntema (1960) who found that there was a progressive drop in the rate of somite formation in snapping turtle embryos developing at temperatures decreasing from 30° to 10°C. Our data would contrast, however, with those of Gabriel (1944) and Dannevig (1950) who found, respectively, that the vertebral numbers of *Fundulus* and plaice (*Pleuronectes platessa*) embryos increased at lower temperatures. Possibly, in the case of these latter authors, investigations were carried out below the critical temperature point of the species.

The inconsistent response of some characteristics to lowered temperatures and the wide range of scale patterns found in our experimental series are readily understandable in view of Tåning's findings that the thermo-sensitive periods of different characters occur at different stages of embryonic development and that temperature responses of individual characters can vary during different thermo-sensitive periods. Since examination of several hundred specimens of *T. e. terrestris* over a period of three years has indicated that nearly all members of the San Francisco peninsula population ovulate within a period of two to three weeks, we are reasonably sure that most of our embryos were exposed to experimental treatment during or immediately subsequent to the first week of development. There was undoubtedly, however, a considerable range in the exact developmental stage achieved by individual litters and embryos at any one specific time during the experiment. Sudden extreme temperature changes or "shocks," which have been shown to be particularly effective in influencing morphogenesis (Tåning, 1952; Lindsey, 1954), could have occurred sporadically at night in our experimental rooms. These would have been experienced simultaneously both by embryos in a relatively thermo-resistant stage and by embryos which were thermo-sensitive for one character or another at that particular time. As Goldschmidt (1945) points out, both unfavorable temperature changes and most mutant genes affect morphogenesis by changing the rate of reaction velocities and altering the sequence of normal developmental processes. Hence phenotypic characteristics which are produced in one species by mutant genes can frequently be experimentally produced in another by temperature shock. Regardless of whether the cause is genetical or environmental, interference with the normally integrated reaction systems necessarily has different morphogenetic effects at different times. At one period of development it may inhibit the production of a determining substance, at another it may delay the production of such a substance until the normally reacting tissues have lost their capacity for response, at still another time it may cause a critical substance to be present in excessive amounts during a particularly sensitive period.

Although, as outlined above, it is possible to offer a plausible explanation of our data solely on the basis of an embryonic sensitivity to thermal variations, one can not entirely ignore the fact that the physiology of the mothers was disturbed by the experimental treatment. Snakes maintained in the cool room did not feed as readily as those in the control room and the gestation period of the former was considerably prolonged. Conceivably, these alterations of the maternal physiology could have secondary teratogenic effects on the embryos. Although it seems unlikely that such secondary effects played an important role in determining the outcome of our experiment, they must be considered as an unknown variable in any analysis of the data. We have considered the possibility that repetition of our experiment with an oviparous species might eliminate the problem of secondary teratogenic maternal influences and thus yield more conclusive data on embryonic responses to chilling. We have come to the conclusion, however, that the results of such a study might be even more difficult to interpret. First, when snake eggs are laid the embryos have usually reached a relatively advanced state of development. Our own data and those of Dr. G. W. D. Hamlett (personal communication) indicate that freshly

laid eggs of several genera of colubrid snakes contain embryos with at least 3½ to 4 coils. We have ascertained by personal examination of stained and cleared specimens that *Heterodon platyrhinos* embryos at the time of egg laying have attained the adult number of body somites although the tail somites are not completely formed. Apparently even in oviparous snakes the exposure of fetuses to teratogenic influences during the earliest stages of development necessitates a simultaneous exposure of the mother. Since such a procedure would allow secondary maternal teratogenic influences to operate during the earliest and, to the belief of most teratologists, the most sensitive stages of development, data obtained on oviparous species would be no more conclusive than those obtained on viviparous species. Secondly, oviparous eggs must be maintained in a mold-free environment under suitable moisture conditions. Inequalities in the amount of moisture in the medium surrounding the eggs or in the humidity of the air in the cool or warm rooms would introduce variables into the experiment which would be difficult to control or evaluate. In a viviparous species, however, presumably moisture conditions would be relatively constant and equivalent in both experimentals and controls.

Since several investigators (see review by Tåning, 1952) have demonstrated that intraspecific genetic differences as well as the thermal environment determine some meristic characteristics of fish embryos, it is unfortunate that the phenotypes of our male parents are unknown. While we were aware from the onset that our experiment might be subject to criticism because of this deficiency, several factors convinced us that it would be impractical to attempt to obtain a series of garter snakes embryos of known parentage for experimental study.

First, we were confronted with the possibility of prolonged sperm survival in the female oviduct. This can be a critical problem in genetical studies on snakes, particularly in some species of Colubrinae and Boiginae where records of survival up to several years have been reported (see review by Fox, 1956). Although the senior author subsequently failed to find sperm in the oviducts or seminal receptacles of either *T. sirtalis* or *T. elegans* at the end of the summer following parturition (Fox, 1956), Blanchard (1942) had demonstrated that sperm of *T. sirtalis* could survive through the winter following fall copulation. Blanchard's data convinced us that if reasonably positive knowledge of the male parentage was to be obtained, female garter snakes would have to be captured during the summer and maintained in isolation in the laboratory until the following spring. This procedure would prevent fall copulations and afford time for sperm from previous copulations to die off.

Several years' study of the reproductive systems of wild and captive garter snakes by the senior author had indicated, however, that unless large outdoor pits or cages similar to those used by Blanchard (1942) were available, such a long period of captivity would prevent successful breeding. He found that although males apparently underwent normal spermatogenic cycles in captivity, the larger follicles of the female ovaries nearly always underwent atresia shortly after being brought into the laboratory. Lack of ovulation and follicular atresia occurred even in many females captured during the peak of the breeding season, at a time when they were swollen with large, palpable follicles. Only two of about 100 female garter snakes he had maintained in the laboratory over winter or for one to two years had been known to ovulate.

Because of the above difficulties, we decided that the present study could only be carried out with expediency if already inseminated females, captured as close to the time of ovulation as possible, were utilized. Although this meant that the exact phenotypes of the male parents were unknown, we had at our disposal data on a large sample of males of the wild population from which the mothers were collected. The chance that the phenotypes of the fathers of our experimental litters differed significantly from those of control litters or males of the natural population is exceedingly small.

In general, thermal variations rather than heredity appeared to play the major role in determining the phenotype of our experimental fetuses. When the female fetuses of the 12 experimental litters were compared to their respective mothers, eight litters were found to deviate markedly from the maternal scale pattern. The remaining four litters showed a closer resemblance to their mothers. Even among the latter litters, however, a few characteristics showed more variation than was present in the mothers. These data reinforce the findings of the previous experiment (Fox, 1948a).

In respect to ventrals and subcaudals there was slight evidence that the maternal phenotype influenced that of her offspring. In calculating correlation coefficients between mothers and male and female fetuses we found that male ventrals and female subcaudals tested to be significant at the .05 level, whereas female ventrals and male subcaudals did not test to be significantly correlated. Since the number of these

scutes closely parallels the vertebral number, it is interesting that Ege (1942) and Tåning (1952) found that heredity played an important role in determining the vertebral number of seatrout embryos although other meristic characters seemed to be determined almost wholly by the thermal environment. Little or no correlation between the snake mothers and fetuses was found when other scale patterns were compared.

Most scute variations seemed to result from the direct teratogenic action of low temperatures on the embryo, and similar action probably produced the harelip occurring in three experimental litters. This malformation also occurred in fetuses of hamsters frozen for short periods during the first week of development (Smith, 1957). In interpreting his data, Smith discussed the possibilities that his results may have been due to maternal hypoxia, freezing of body water with a consequent increased concentration of solutes in the medium surrounding the embryo, placental damage, or direct embryonic arrest. Our data lend support to his hypothesis of direct embryonic arrest.

Examination of the wild population of *Thamnophis elegans terrestris* from our collecting grounds in San Mateo County, California, indicates that even under natural thermal conditions the experimental species exhibits an unusually wide range of scutellation patterns. When compared to another species, *Thamnophis sirtalis tetrataenia,* which inhabits the same area, it was found to be much less constant in respect to a number of scale counts. In *T. e. terrestris,* for example, the most common scale row formula (19-21-17) is present in only 65.5 per cent. of the population whereas in *T. s. tetrataenia* the characteristic pattern (19-19-17) occurs in 94.5 per cent. of the specimens. Similarly, while a lower labial formula of 10-10 occurs in 85.3 per cent. of *T. s. tetrataenia* and an undivided preocular in 100 per cent., in *T. e. terrestris* only 75.0 per cent. have a lower labial formula of 10-10 and the preocular is divided in 11.3 per cent. of the specimens.

This natural variation which occurs in the experimental species, coupled with the fact that there was a very high mortality of experimental fetuses in the first experiment (Fox, 1948a), has led one geneticist to suggest to the senior author that the lowered scale counts he obtained in the 1947 series were due to selection rather than to direct thermal influences. It was postulated that given a wide range of genetic variation within a litter, only those fetuses with low scale counts might have been able to survive the experimental treatment. We find this hypothesis untenable for several reasons. First, the mortality of experimental fetuses in the present experiment was not high enough to allow for the selective removal of an appreciable number of fetuses with normal or higher scale counts. Secondly, many of the extreme scale counts and patterns which appeared in our experimental series have never been seen either in the control litters, in the wild population or in litters of recently captured females. One must conclude, therefore, that they do not normally occur or are all selectively eliminated before birth under normal temperature conditions. The fact that our data indicate that there is no appreciable prenatal mortality in the litters of control or wild females argues strongly against the latter possibility. Lastly, the hypothesis is based on the assumption that the variable scale counts of *T. e. terrestris* are based solely on genetical differences. Since our experiments have demonstrated that one of the chief effects of cold exposure during gestation is the production of a wide range of scutellation patterns, it seems possible that much of the natural variation which occurs in the population from which the experimental snakes were taken may be due to the thermal conditions of its habitat. Certainly, in the cool, fog-bound coastal hills which it inhabits there is ample opportunity for periodic chilling during embryonic development. The fact that *T. sirtalis tetrataenia,* which is exposed to identical thermal variations, does not exhibit a similiar variability, does not necessarily negate this possibility. A genetic difference in susceptibility to thermal variations could explain this difference between the two species.

Lest taxonomists, as a result of our experiment, become unduly concerned over the possibility that non-genetical variations of usually reliable taxonomic characteristics may have resulted in erroneous recognition of subspecies or species of reptiles, we should like to state that we doubt whether many such errors have occurred. In spite of all the induced modifications of scute patterns in *T. e. terrestris,* the experimental young were still recognizable as to subspecies on the basis of color and pattern although many of them would not fit "keys" to the genus *Thamnophis* and a few would even "key out" to different genera. The limitations of the artificial "keys" are not important, however, when a complete description of a "kind" of animal is concerned. Perhaps the main thing that this study might emphasize for the taxonomist is the desirability of large samples when describing a new form. It might further lead one to anticipate a fair amount of variation in poikilothermic animals, particularly in those populations from areas that are fog-bound or

cool where there is an opportunity for chilling during embryonic development.

Summary

1. Gravid garter snakes of the subspecies *Thamnophis elegans terrestris* were maintained at temperatures fluctuating from 65°-85°F (cool room, experimentals) and 75°-95°F (warm room, controls) throughout most of the gestation period.

2. The experimental fetuses showed a very wide range of variation in scutellation patterns. The principal effect of lower temperatures on ventrals, subcaudals, labials and scale rows around the body was a reduction in number. Other scutes divided or fused irregularly. Of special interest was an unusual incidence of divided anal plate in five litters and harelip in three litters.

3. Only in respect to the ventrals and subcaudals was there a possible correlation between the scale patterns of the fetuses and their mothers. The data show that considerable phenotypic variation is possible within the limits of the genotype of a population.

4. In all probability, the nightly chilling of the embryos during early critical periods of morphogenesis was the most important teratogenic factor to which they were exposed. Since thermal variations behave like most mutant genes in that they interfere with the normal sequence of development processes (Goldschmidt, 1945), lowered temperatures produced scale patterns which are not only atypical for the subspecies but atypical for the genus.

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